Cervical Specimens Collected in Liquid Buffer Are Suitable for Both Cytologic Screening and Ancillary Human Papillomavirus Testing

Mark E. Sherman, M.D.[1,2]
Mark H. Schiffman, M.D., M.P.H.[2]
Attila T. Lorincz, Ph.D.[3]
Rolando Herrero, M.D., Ph.D.[4]
Martha L. Hutchinson, M.D., Ph.D.[5]
Concepcion Bratti, M.D.[6]
David Zahniser, Ph.D.[7]
Jorge Morales, M.D.[8]
Allan Hildesheim, Ph.D.[2]
Kay Helgesen, M.S.[9]
Deidra Kelly, B.A.[10]
Mario Alfaro, M.D.[8]
Fernando Mena, M.D.[8]
Ileana Balmaceda, M.D.[6]
Laurie Mango, M.D.[11]
Mitchell Greenberg, M.D.[12]

[1] Department of Pathology, the George Washington University Medical Center, Washington, DC.

[2] Division of Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland.

[3] Digene Corporation, Silver Spring, Maryland.

[4] International Agency for Research on Cancer, Lyon, France.

[5] Department of Pathology, New England Medical Center, Boston, Massachusetts.

[6] Ministerio de Salud, San Jose, Costa Rica.

[7] Cytyc Corporation, Boxborough, Massachusetts.

[8] Caja Costarricense de Seguro Social, San Jose, Costa Rica.

[9] Information Management Services, Silver Spring, Maryland.

[10] Department of Pathology, the Johns Hopkins Hospital, Baltimore, Maryland.

[11] Neuromedical Systems Inc., Suffern, New York.

[12] Department of Obstetrics and Gynecology, Graduate Hospital, Philadelphia, Pennsylvania.

BACKGROUND. Several new techniques have been developed to improve the sensitivity of cervical carcinoma screening and reduce equivocal cytologic diagnoses referred to as atypical squamous cells of undetermined significance (ASCUS). This study evaluates the effectiveness of combining two newly introduced diagnostic techniques: preparation of thin-layer cytologic slides from ThinPrep liquid buffer and selected Hybrid Capture testing for human papillomavirus (HPV) DNA. Because HPV DNA detection has been strongly associated with the presence of a cervical carcinoma precursor (''squamous intraepithelial lesion,'' or SIL), HPV testing might be useful for identifying women with ASCUS who have an underlying SIL.

METHODS. Two hundred specimens demonstrating diverse cervical abnormalities were selected from a prospective population-based study of 9174 women conducted in Costa Rica. The entire cohort had been screened with conventional cervical smears; ThinPrep slides made from liquid buffer; PAPNET, a computerized slide reading system; and Cervicography. Patients with any abnormal screening test were referred for colposcopy, punch biopsy, and loop excision of cases with high grade cytologic abnormalities not explained by punch biopsy. For this investigation, the results of ThinPrep cytology and HPV testing alone and in combination

Presented in part at the International Papillomavirus Workshop, Quebec, Canada, July 1995, and abstracted in the Proceedings of the American Society of Cytopathology Meeting, New York, NY, November 1995 (*Acta Cytol* 1995;39:996).

Supported in part by National Cancer Institute contracts 163-MQ-440433, NO1-CP-21081, and NO1-CP-31061.

Dr. Sherman has served as an investigator on research contracts awarded by Neuromedical Systems to Johns Hopkins University and by Cytyc and Upjohn to George Washington University; Dr. Lorincz is Vice President and Scientific Director, Digene Corporation; Dr. Hutchinson has served as a consultant and contract investigator for Ciba Corning, CompuCyte, Cytyc, Matritech, and Oncometrics and is a stockholder in Cytyc, Neopath, and Neuromedical Systems; Dr. Zahniser is Vice President and Director of Scientific Affairs at Cytyc Corporation; Dr. Mango is Vice President and Scientific Director, Neuromedical Systems, Inc.; Dr. Greenberg is a paid evaluator of Cervigrams for National Testing Laboratories and is an employee of Omnia, which has consulting relationships with Digene, Cytyc, 3M, and Ethicon. The human papillomavirus testing for this study was performed at Digene and Johns Hopkins and paid for with funds awarded to Dr. Sherman through an NCI contract, as noted in an earlier footnote.

The authors thank Ms. Karen Plowden and Ms. Linda Reynolds for providing excellent technical consultation. They appreciate the donation of LEEP equipment by Utah Medical Products, Midvale, Utah. They also thank Dr. Keerti Shah and Ms. Angela Jensen at the Johns Hopkins School of Public Health for performing polymerase chain reaction–based testing for human papillomavirus on selected specimens.

Dr. Sherman's current address: The Johns Hopkins Hospital, Baltimore, MD. Dr. Hutchinson is currently at Women and Infants' Hospital, Providence, RI. Dr. Greenberg is currently with Omnia, Inc., Philadelphia, PA.

Address for reprints: Mark E. Sherman, M.D., Department of Pathology, the Johns Hopkins Hospital, 600 N. Wolfe Street, Room 406, Baltimore, MD 21287.

Received September 20, 1996; revision received December 8, 1996; accepted December 8, 1996.

were compared with the final diagnoses, with an emphasis on the detection of carcinoma and high grade SIL.

RESULTS. The 200 subjects studied included 7 women with a final diagnosis of carcinoma, 44 with high grade SIL, 34 with low grade SIL, 51 with a variety of equivocal diagnoses, and 64 with normal diagnoses. A ThinPrep cytologic diagnosis of SIL or carcinoma was made in 39 (76%) of the 51 women with final diagnoses of high grade SIL or carcinoma. Hybrid Capture testing detected carcinoma-associated types of HPV DNA in 100% of women with carcinoma, 75% with high grade SIL, 62% with low grade SIL, 20% with equivocal final diagnoses, and 12% of normal women. If colposcopy referral had been limited to women with a ThinPrep diagnosis of SIL or a diagnosis of ASCUS associated with the detection of carcinoma-associated HPV DNA from the same vial, 100% of women with carcinoma and 80% with high grade SIL would have been examined. To achieve this high sensitivity in the entire population of 9174 women would have required the referral of about 7% of the population. The combined screening strategy would have performed marginally better than optimized conventional screening with referral of any abnormal cytology (ASCUS and above).

CONCLUSIONS. A cervical carcinoma screening technique which uses a single sample for cytopathology and HPV testing to triage equivocal diagnoses may be promising if it proves to be cost-effective. ***Cancer (Cancer Cytopathol)*** **1997;81:89–97.**

KEYWORDS: cervix, Bethesda System, carcinoma, human papillomavirus, hybridization, atypical squamous cells of undetermined significance.

The use of Papanicolaou smears to screen for cervical carcinoma is widely recognized as an effective public health measure. Nonetheless, erroneous and equivocal diagnoses pose important clinical problems.[1–3] The most common problem relates to the equivocal cytologic diagnoses, referred to as atypical squamous cells of undetermined significance (ASCUS). Because ASCUS diagnoses comprise 5–10% of smears in many laboratories, routine referral of women with ASCUS for colposcopy is impractical. Better screening methods are needed to clarify the ASCUS diagnostic category and to improve the overall accuracy of cervical carcinoma screening. Approaches that are under consideration include improved methods of cytologic preparation and computer-assisted slide review;[4] visual techniques, such as Cervicography (National Testing Laboratories, Fenton, MO);[5] and testing for underlying oncogenic human papillomavirus (HPV) infection using DNA diagnostic methods.[6–9]

Previous studies have demonstrated that the detection of carcinoma-associated types of HPV correlates with the presence of a cervical carcinoma precursor (squamous intraepithelial lesion, or SIL) or invasive carcinoma.[10–14] Specifically, in previous studies of ASCUS using the Hybrid Capture tube test (Digene Corporation, Silver Spring, MD), an HPV test approved by the U.S. Food and Drug Administration, the detection of HPV DNA appeared to identify women with ASCUS who harbored an underlying SIL. In particular, HPV testing permitted the sensitive identification of women with high grade SIL, the immediate precursor of carcinoma. Recognition that the vast majority of low grade SILs spontaneously regress has focused screening efforts on the identification and treatment of women with high grade SIL.[15]

To date, HPV testing using Hybrid Capture has required that women diagnosed with ASCUS return for a second clinical visit to provide another cytologic sample for HPV testing. A more cost-effective protocol would entail the collection at the initial visit of specimens for both cytologic diagnosis and HPV testing if indicated.[8,11] The development of a single-collection method suitable for both cytologic diagnosis and virologic testing would be the ideal approach.

Recently, a new cytologic technique, in which exfoliated cervical cells are collected in liquid buffer (PreservCyt, Cytyc, Boxborough, MA) and transported to the laboratory for preparation as thin-layer slides (ThinPrep, Cytyc Corporation, Boxborough, MA), has received Food and Drug Administration approval for clinical use. ThinPrep cytology has demonstrated equivalent or increased detection of SIL and carcinoma in comparison with conventional Papanicolaou smears.[16–19] Because the production of a ThinPrep uses only a fraction of the cells collected in PreservCyt buffer, the residual cells re-

maining in the vial are available for ancillary studies, including HPV DNA testing.

In this study, we evaluate the feasibility of a screening strategy in which single specimens collected in PreservCyt are used for both cytopathologic diagnosis and HPV testing.

MATERIALS AND METHODS

Case Selection

Cases were selected from a National Cancer Institute–sponsored screening study of 10,738 randomly selected women residing in Guanacaste Province, Costa Rica, an area with a persistently high incidence of cervical carcinoma. Participation rates exceeded 90%.[20] Consenting, nonvirgin cohort members (n = 9174) were screened between June 1993 and December 1994 with multiple cytologic and visual methods (see below).

The 200 cases chosen for this study were selected while enrollment was proceeding, before final case definitions (see below) were available. To capture the full spectrum of pathology, a stratified random sample was taken of the available ThinPrep cytopathologic diagnoses in August 1994. We included virtually all ThinPrep diagnoses of invasive carcinoma (n = 5), high grade SIL (n = 31), low grade SIL (n = 39), and ASCUS (n = 63) known by that time, and also chose a random sample of ThinPrep-negative cases with positive (n = 35) or negative (n = 26) colposcopy and biopsy. One woman with an unsatisfactory ThinPrep was also included. The false-negative and unsatisfactory ThinPrep cases were oversampled to determine the results of ancillary HPV testing in cases where ThinPrep cytology apparently had failed.

Clinical Procedures

Subjects provided informed consent at enrollment, and pregnant women were deferred until 3 months after delivery. A pelvic examination was performed by a small group of specially trained nurses who referred subjects with gross lesions immediately to colposcopy.

Exfoliated cervical cells collected with a Cervex brush (Unimar, Wilton, CT) were used to prepare a conventional smear that was spray-fixed with Pap Perfect (Medscand, Hollywood, FL). Residual cells on the Cervex brush were rinsed in PreservCyt and shipped at ambient temperature to the U.S. The cell suspensions were used to prepare ThinPrep slides and then stored at room temperature.

A second sample of exfoliated cervical cells was obtained with a Dacron swab and placed in Specimen Transport Medium (STM, Digene Corporation, Silver Spring, MD) for conventional HPV testing. The STM sample was frozen at −30 °C in the field, then stored at −70 °C at a central facility in Costa Rica prior to shipment to the U.S. on dry ice.

After collection of the cytologic specimens, the cervix was rinsed twice with 5% acetic acid, and two Cervigrams (photographic images of the cervix) were obtained (National Testing Laboratories, Fenton, MO).

Laboratory Testing Methods

Conventional cervical smears were stained with a modified Papanicolaou method in Costa Rica, screened, and diagnosed by a single expert Costa Rican cytopathologist (M.A.) using the Bethesda System.[1] Diagnoses made with the Bethesda System included normal/benign cellular changes (negative), ASCUS, low grade SIL, high grade SIL, and carcinoma. The smears were then transported to the U.S. and examined with the PAPNET system (Neuromedical Systems, Inc., Suffern, NY), a neural network–based automated screening system.[21–23] Smears in which potential abnormalities were detected by a trained cytotechnologist (D.K.) using PAPNET were rescreened with conventional microscopy and then forwarded to a pathologist (M.E.S.) for final diagnosis, again using the Bethesda System.

Cervical cells suspended in PreservCyt were made into cytologic slides containing a 20 mm-diameter circular deposit of cells (ThinPreps) using the ThinPrep processor.[16–19] ThinPreps are prepared by placing a PreservCyt vial and a slide onto the processor, which mixes the specimen as cells are drawn onto a polycarbonate filter by suction. When sufficient cells have been removed to prepare a thin-layer slide, the cells are transferred from the filter to the slide and the slide is immersed in ethanol. The ThinPreps were stained by a modified Papanicolaou method, screened by cytotechnologists, and diagnosed according to the Bethesda System in the U.S. by one cytopathologist (M.L.H.). The PreservCyt vials were stored at room temperature throughout.

Cervigrams were graded by an expert evaluator (M.G.) in the U.S. as unsatisfactory, normal, atypical, or positive. Positive Cervigrams were classified as P0 (probably normal but rule out significant lesion), P1 (low grade squamous intraepithelial lesion), P2 (high grade squamous intraepithelial lesion), and P3 (carcinoma).

Subjects found to have cytologic abnormalities (ASCUS, SIL, or carcinoma) by any of the three cytologic techniques or a positive Cervigram were referred to a gynecologist-colposcopist (J.M.) for examination, with biopsy of visible lesions. A 5% sample of the remaining women was also referred for colposcopy as controls. The complete absence of significant findings in the controls confirmed that the screening protocol detected virtually 100% of lesions detectable by colposcopy and biopsy.

Colposcopic Evaluation

At colposcopy, the appearance of the cervix was systematically described and recorded for quality assurance purposes using digital imaging (Denvu, RMC, Tucson, AZ). Biopsies were fixed in 10% buffered formalin, paraffin embedded, and prepared as conventional histopathologic slides stained with hematoxylin and eosin. The biopsies were diagnosed by a Costa Rican pathologist (F.M.) using the Bethesda System and then reviewed in the U.S. (by M.E.S.). In cases of disagreement, the U.S. diagnosis was used for study purposes. However, final treatment decisions were made by the Costa Rican gynecologist (J.M.) based on all available data. Women with histologically confirmed high grade SIL or two cytologic reports of high grade SIL were treated, usually with large loop excision of the transformation zone (LEEP) or cold knife cone. Women with a single cytologic diagnosis of high grade SIL that was not histologically confirmed underwent cone biopsy or LEEP if the cytologic abnormality was confirmed to be high grade SIL on review, a lesion was visible by colposcopy and there were no contraindications or refusal of treatment. If LEEP was not performed, the patients were referred through the Costa Rican Social Security system for additional follow-up. Pathologic findings in LEEP and hysterectomy specimens were reviewed in the U.S. (by M.E.S.) when these specimens were available as part of assigning final case diagnoses.

Final Case Diagnoses

Final case diagnoses were assigned by a panel of the collaborating clinicians and pathologists based on the integrated interpretation of all available screening and diagnostic tests. A variant of the Bethesda System was used for categorization and statistical analysis. Diagnoses were assigned without knowledge of HPV test results. All cases with final diagnoses of invasive carcinoma (''Final-carcinoma'') and high grade SIL (''Final-HSIL'') were histopathologically confirmed. Low grade SILs were initially divided into histologically confirmed diagnoses and those based on careful cytopathology review; but for the purposes of this study, the results have been combined as ''Final-LSIL.'' Equivocal final case diagnoses (''Final-equivocal'') reflected various combinations of equivocal screening test results, including a single cytologic diagnosis of low grade SIL (conventional or ThinPrep), a positive Cervigram without cytologic or histologic abnormality, and cases with uncertain final status on review of all data. Final case diagnoses of normal (''Final-normal'') included women with entirely normal screening tests as well as women referred for colposcopy because of ASCUS cytology but considered normal on final review.

Human Papillomavirus Testing

The suitability of PreservCyt samples for HPV testing was evaluated by comparing the Hybrid Capture results obtained using these samples with those obtained independently (and masked) using specimens collected concurrently in 1 mL of STM, the Digene buffer approved by the U.S. Food and Drug Administration for HPV testing.

Following the preparation of a ThinPrep slide, PreservCyt samples were prepared for HPV testing by centrifuging a 6 mL aliquot at 10,000 g for 10 minutes in a 15 mL conical tube. The resulting cell button was resuspended in 1 mL of supernatant and then spun for 5 minutes in a microcentrifuge. The pellet was drained, leaving about 10 μL of liquid, suspended in 180 μL of a 2:1 mixture of STM:Denaturation Reagent, and incubated for 45 minutes in a 65 °C water bath.

STM specimens were prepared for Hybrid Capture testing by adding one drop of proteinase solution (''blue juice'') followed by incubation for 5 minutes in a 37 °C water bath. Then specimens were placed on a shaker for 30 minutes at 1100 rpm. An aliquot of 650 μL of each sample was removed and added to tubes containing 325 μL of Denaturation Reagent, then placed on a shaker for 1 minute at 1100 rpm. Specimens were then denatured for 45 minutes at 65 °C.

Separate 150 μL aliquots of the denatured PreservCyt and STM samples were hybridized with cocktails of full length HPV RNA probes recognizing low risk (6, 11, 42, 43, 44) and carcinoma-associated (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58) viral types using the Hybrid Capture tube test.[24] Hybrids composed of HPV DNA and type specific RNA probes were captured on the surface of tubes coated with an antibody recognizing DNA-RNA hybrids. Detection of hybrids was achieved with a second antibody conjugated to alkaline phosphatase followed by addition of an enzyme substrate. The light emitted in positive assays was measured with a luminometer in relative light units (RLUs) proportional to the amount of HPV DNA in the specimen. The RLUs of the specimens were divided by the mean RLU of three positive controls. Specimens in which this ratio was $\geq$1.0 were considered HPV-positive; samples with ratios $<$1.0 were considered negative.

PCR-Based HPV Testing of Selected Cases

After the completion of the initial analysis, polymerase chain reaction (PCR)–based studies were performed on 50 remasked specimens collected in STM to determine the possible reasons for the failure of Hybrid Capture to detect HPV DNA in some women diagnosed with Final-HSIL. The specimens tested by PCR included 2 separate samples obtained from each of the 11 women with a diagnosis of Final-HSIL and a negative Hybrid Capture HPV test (the Hybrid Capture–negative specimen obtained at enrollment and

TABLE 1
Comparison of ThinPrep Cytologic Diagnoses with Final Case Diagnosis

ThinPrep diagnosis	Final case diagnosis					
	Final-normal	Final-equivocal	Final-LSIL	Final-HSIL	Final-carcinoma	Total
Unsatisfactory	1 (1.6%)	0	0	0	0	1 (0.5%)
Negative	33 (51.6%)	21 (41.2%)	3 (8.8%)	4 (9.1%)	0	61 (30.5%)
ASCUS	30 (46.9%)	19 (37.3%)	6 (17.6%)	6 (13.6%)	2 (28.6%)	63 (31.5%)
LSIL	0	8 (15.7%)	23 (67.6%)	8 (18.2%)	0	39 (19.5%)
HSIL	0	2 (3.9%)	2 (5.9%)	23 (52.3%)	4 (57.1%)	31 (15.5%)
Carcinoma	0	1 (2.0%)	0	3 (6.8%)	1 (14.3%)	5 (2.5%)
Total	64 (100%)	51 (100%)	34 (100%)	44 (100%)	7 (100%)	200 (100%)

ASCUS: atypical squamous cells of undetermined significance; SIL; squamous intraepithelial lesion; LSIL: low grade SIL; HSIL: high grade SIL.

TABLE 2A
Detection of HPV DNA by Hybrid Capture Tube Test in PreservCyt versus STM

	No. of cases (%)[a]		
	STM neg.	STM pos.	Total
PreservCyt neg.	103 (51.5)	7 (3.5)	110 (55.0)
PreservCyt pos.	24 (12.0)	66 (33.0)	90 (45.0)
Total	127 (63.5)	73 (36.5)	200 (100.0)

HPV: human papillomavirus; STM: specimen transport medium; neg: negative; pos: positive.
[a] Data are based on a total of 200 cases.

TABLE 2B
Detection of Carcinoma-Associated HPV DNA by Hybrid Capture Tube Test in PreservCyt versus STM

	No. of cases (%)[a]		
	STM neg.	STM pos.	Total
PreservCyt neg.	115 (57.5)	6 (3.0)	121 (60.5)
PreservCyt pos.	16 (8.0)	63 (31.5)	79 (39.5)
Total	131 (65.5)	69 (34.5)	200 (100.0)

HPV: human papillomavirus; STM: specimen transport medium; neg: negative; pos: positive.
[a] Data are based on a total of 200 cases.

an additional, untested one collected at colposcopy approximately 1–2 months later). For comparison, we tested by PCR the enrollment specimens obtained from 13 women with Final-LSIL who had a negative Hybrid Capture HPV test as well as 15 enrollment specimens obtained from Hybrid Capture–negative, Final-normal women.

PCR testing was performed using a sensitive L1 consensus primer method capable of detecting over 25 different HPV types. This method is described in detail elsewhere.[25–27] Because the STM specimens had been manipulated less than the PreservCyt vials, the former were used for PCR analysis to minimize the risk of a false-positive result due to specimen contamination.

Data Analysis

The diagnoses of ThinPreps were compared with the subsequent final case diagnoses. The results of Hybrid Capture HPV testing of PreservCyt samples were compared with the results obtained independently with the STM specimens and with the final case diagnoses. The hypothetical effectiveness of a cervical carcinoma screening strategy was also assessed; in this strategy cells collected in PreservCyt would be used for both ThinPrep cytopathologic diagnosis and HPV testing of ASCUS. For these analyses, the final case diagnosis (reflecting the three cytologic screening methods and histopathology) was used as the gold standard. Finally, we compared the detection of HPV by PCR, in cases of SIL in which the Hybrid Capture test was negative, with control women who were cytologically normal and had a negative Hybrid Capture test. Comparisons were performed using standard contingency table analyses.

RESULTS

ThinPrep Diagnoses versus Final Case Diagnoses

Diagnoses of satisfactory ThinPreps and final case diagnoses agreed exactly in 99 (49.5%) of 199 cases (Table 1). A ThinPrep diagnosis of ASCUS or worse was made in 47 (92.2%) of 51 women with a final diagnosis of Final-HSIL or Final-carcinoma. A ThinPrep diagnosis of SIL or carcinoma was made in 39 (76.5%) of these 51 cases.

HPV DNA Detection by Hybrid Capture in PreservCyt versus STM

HPV DNA was detected in 90 (45.0%) of 200 PreservCyt samples as compared with 73 (36.5%) of 200 corre-

TABLE 3
Detection of HPV DNA in PreservCyt Using Hybrid Capture, by Final Case Diagnosis

	Final case diagnosis					
HPV DNA	Final-normal (n = 64)	Final-equivocal (n = 51)	Final-LSIL (n = 34)	Final-HSIL (n = 44)	Final-carcinoma (n = 7)	Total (n = 200)
Any type	12 (18.8%)	14 (27.4%)	24 (70.6%)	33 (75.0%)	7 (100%)	90 (45.0%)
Carcinoma-associated	8 (12.5%)	10 (19.6%)	21 (61.8%)	33 (75.0%)	7 (100%)	79 (39.5%)

HPV: human papillomavirus; LSIL: low grade squamous intraepithelial lesion; HSIL: high grade squamous intraepithelial lesion.

sponding STM specimens (Table 2). Overall, HPV DNA detection was concordant in 169 samples (84.5%). Similarly, carcinoma-associated HPV types were identified in 79 (39.5%) PreservCyt specimens as compared with 69 (34.5%) STM samples, with overall agreement for the detection of carcinoma-associated types in 178 subjects (89.0%). HPV positivity was higher with PreservCyt than with STM for both overall HPV positivity ($P = 0.004$, McNemar's test) and the carcinoma-associated type group ($P = 0.06$).

Carcinoma-associated HPV types accounted for the great majority of HPV DNA detected in both PreservCyt and STM. Detection of low risk HPV types was not significantly associated with disease status (data not shown) and will not be discussed further.

Detection of HPV DNA by Hybrid Capture in PreservCyt versus Final Case Diagnosis

As shown in Table 3, carcinoma-associated HPV types were detected in PreservCyt in 100.0% of women with Final-carcinoma, 75.0% with Final-HSIL, and 61.8% with Final-LSIL, as compared with 19.6% of women with Final-equivocal diagnoses and 12.5% of Final-normal women (P for trend <0.0001). Results obtained with STM samples showed similar strong trends but somewhat reduced HPV positivity in all categories of final diagnosis (data not shown).

HPV Testing of PreservCyt Samples with Hybrid Capture to Clarify ThinPrep Diagnoses of ASCUS

Carcinoma-associated types of HPV DNA were detected in 16 (25.4%) of 63 PreservCyt samples obtained from women with a ThinPrep diagnosis of ASCUS (Table 4). Women with an underlying final diagnosis of SIL or carcinoma were more likely to be HPV-positive than those with Final-normal or Final-equivocal diagnoses (P for trend = 0.05 for data presented in Table 4). Notably, in women with a ThinPrep diagnosis of ASCUS, carcinoma-associated HPV types were identified in both women with an underlying carcinoma (Final-carcinoma) but in only one of six women with Final-HSIL.

Summary of Screening with Both ThinPrep Cytology and Hybrid Capture

Extrapolating the preceding results to the population-based, 9174-woman cohort demonstrates the potential utility of combining ThinPrep cytology with Hybrid Capture testing of residual cells in the collection vial. Specifically, if all women with a ThinPrep diagnosis of SIL or carcinoma and those with a diagnosis of ASCUS associated with the detection of carcinoma-associated HPV DNA had been referred for colposcopy, about 7% of women in Guanacaste would have been referred. Based on the diagnosis of the ThinPrep alone, 0.1% of the population would have been referred for a diagnosis of carcinoma, 1.6% for high grade SIL, and 3.2% for low grade SIL. Thus, ThinPrep cytology by itself would have resulted in a referral rate of 4.9%. In addition, 7.1% of women had a ThinPrep diagnosis of ASCUS. Because a carcinoma-associated HPV type was detected in about one-fourth of these women, an additional 1.8% of the population would have been referred based on the combined result of ASCUS cytology and a positive HPV test. In total, 6.7% of the population would have been referred for colposcopy based on either definitely abnormal cytology (SIL or carcinoma) or equivocal cytology associated with carcinoma-associated HPV. An estimated 100% of carcinomas and 80% of HSILS would have been detected.

In comparison, in the same population of 9174 women, conventional cytology detected about 92% of carcinomas and 76% of high grade lesions, referring about 7% of the population for a Pap smear diagnosis of ASCUS, SIL, or carcinoma (primary data not shown).

PCR-Based Testing of Selected STM Specimens

HPV DNA was detected by PCR in 40.0% of the 10 evaluable enrollment specimens obtained from women with Final-HSIL in whom the Hybrid Capture test performed on the PreservCyt sample was negative. (The addition of a second specimen from colposcopy raised the total positivity in 11 evaluable women to 63.6%.) In comparison, 7 (53.8%) of 12 samples with amplifiable DNA obtained from women with Final-

TABLE 4
Detection by Hybrid Capture of Carcinoma-Associated HPV Types in PreservCyt, According to Final Case Diagnosis, among Women with a ThinPrep Diagnosis of ASCUS

	Final case diagnosis					
HPV DNA	Final-normal (n = 30)	Final-equivocal (n = 19)	Final-LSIL (n = 6)	Final-HSIL (n = 6)	Final-carcinoma (n = 2)	Total (n = 63)
Carcinoma-associated	5 (16.7%)	5 (26.3%)	3 (50.0%)	1 (16.7%)	2 (100%)	16 (25.4%)

HPV: human papillomavirus; ASCUS: atypical squamous cells of undetermined significance; LSIL: low grade squamous intraepithelial lesion; HSIL: high grade squamous intraepithelial lesion.

LSIL and 3 (20.0%) of 15 obtained from women with a diagnosis of Final-normal tested positive only by PCR. The types of HPV found by PCR in women with Final-HSIL and a negative Hybrid Capture test included types 16, 33, 51, and 58, as well as types not included in the Hybrid Capture test.

DISCUSSION

This study demonstrates the potential utility of a new cervical carcinoma screening approach in which specimens collected in liquid buffer are used for both cytopathologic diagnosis of thin-layer preparations and HPV testing if indicated. If colposcopy had been limited to the 7% of women in Guanacaste with ThinPreps showing SIL/carcinoma, or ASCUS associated with the detection of a carcinoma-associated HPV type in the PreservCyt vial, the referrals would have included 100% of women with Final-carcinoma, 80% with Final-HSIL, and 82% with Final-LSIL.

These sensitivity figures compare quite favorably with average performance data documented in conventional cervical cytologic screening programs in the United States and Europe.[28–30] However, among the 9174 women screened in our Guanacaste project, the ThinPrep-HPV combination performed only marginally better than the conventional Papanicolaou smear, because the sensitivity and specificity of conventional cytology was unusually high. As a possible explanation for the superior performance of the conventional smear in Guanacaste as compared with results reported in the literature, our Guanacaste project relied on just a few highly-trained, expert clinicians and pathologists. Several rounds of training and monitoring were conducted. Thus, errors of cell collection, fixation, staining, screening, and interpretation were undoubtedly reduced as compared with those occurring in routine practice. Perhaps some unknown characteristic of the population also contributed to the unusual success of the screening. Whatever the explanation, it appears that the value of the ThinPrep-HPV combination, or any technique introduced as a possible replacement or adjunct to the Papanicolaou smear, must be compared not only with historical screening data, but also with the possibility of optimized conventional screening.

It is quite probable that the optimal cervical carcinoma screening method will differ by regional capabilities. For settings favoring a single, reliable clinical collection that minimizes equivocal diagnoses and the need for repeated patient appointments, combined cytopathologic diagnosis and selected HPV testing from PreservCyt specimens is an interesting option.

Most previous studies of Hybrid Capture have also suggested that testing for carcinoma-associated types of HPV might be useful in determining which women with ASCUS require colposcopy. Cox et al. reported that repeat cytology and HPV testing, in combination, provided sensitive colposcopy triage in college-age women with ASCUS while reducing referrals by half.[11] Wright et al. achieved sensitive colposcopy triage using combined testing, but concluded that obtaining the sample for HPV testing at the initial screening visit rather than requiring a repeat visit to obtain another sample was necessary for cost-effectiveness.[14]

The single-collection method described in this study offers several advantages over collecting a separate sample for HPV testing. First, only one sampling procedure is needed. Second, the quality and cellularity of the specimen used for HPV testing are known from its cytologic appearance and the volume of fluid required to prepare a ThinPrep. Third, this approach links HPV testing to a specific cytologic diagnosis, thereby focusing HPV testing. Finally, our results indicate that Hybrid Capture testing performed on PreservCyt yields results at least as sensitive as testing of STM samples. Similarly, previous studies have demonstrated that ThinPreps and smears prepared from split samples are diagnostically equivalent.[16–19] In summary, the single-collection method combines logistic efficiency with sensitive virologic and cytologic techniques. Efforts are now underway to make the combined procedure semiautomatic, which would reduce cost.

It is noteworthy that Hybrid Capture testing was

positive in both women with ThinPrep diagnoses of ASCUS whose final diagnosis was carcinoma, but was negative in five of six women with a ThinPrep diagnosis of ASCUS and Final-HSIL. The significance of this finding is unclear, given that other investigations have demonstrated good sensitivity for detection of underlying high grade SIL in women with cytologic diagnoses of ASCUS.[11] Inadequate sampling of the lesion or failure of the diagnostic cells to exfoliate due to the intrinsic properties of the tissue are possible explanations for these five missed cases. It is noteworthy that four of the five missed cases were diagnosed as high grade SIL on the conventional smears, whereas the residual cells placed into PreservCyt led to ASCUS diagnoses with ThinPrep and DNA negativity with Hybrid Capture, suggesting that diagnostic cells in the specimen collection may have been scant. Theoretically, rinsing the sampler in the PreservCyt vial without initially preparing a smear could improve the sensitivity of both ThinPrep cytopathology and Hybrid Capture HPV testing by increasing the number of abnormal cells available for study.

The hypothesis that Hybrid Capture testing is affected by sampling may also be supported by the fact that in several of the women with Final-HSIL deemed HPV-negative by Hybrid Capture, PCR testing detected HPV types included in the Hybrid Capture kit. In these missed cases, the amount of HPV DNA in the PreservCyt vial was apparently below the level of Hybrid Capture detection.

Cells suspended in PreservCyt may be suitable for HPV testing using PCR-based methods,[31] but the specificity and positive predictive value of PCR-based testing for colposcopy triage is unknown. Although PCR testing raises concerns about specimen contamination leading to false-positive results, promising efforts to develop PCR-based HPV tests suitable for clinical use are being made (P. Gravitt, unpublished data). At the same time, a new microplate version of Hybrid Capture that uses an increased number of type specific probes and has increased analytic sensitivity may yield higher sensitivity of HPV detection than was realized in this study (M. H. Schiffman, unpublished data).

Implementation of the new cervical carcinoma screening approach described in this report would permit clinicians to focus on specimen collection while allowing laboratories to concentrate on slide preparation, diagnosis, and ancillary testing. In the proposed model, clinicians would place the entire sample of exfoliated cervical cells immediately into a buffer for transport to the laboratory rather than preparing a smear. In the laboratory, a thin-layer slide would be prepared, Papanicolaou-stained, screened by cytotechnologists, and interpreted in the usual manner. Vials containing the residual samples would be stored temporarily at room temperature pending cytologic diagnosis. In the majority of cases, negative, SIL, and carcinoma diagnoses would be reported and the vials would be discarded. Technically inadequate thin-layer slides could be repeated. In cases with ASCUS diagnoses, the vials would be tested for carcinoma-associated HPV types. Women with ASCUS associated with carcinoma-associated HPV DNA would be referred for colposcopy, whereas in the majority of patients with ASCUS, colposcopy could be avoided with added confidence.

Although the prospect of greatly decreasing the numbers of inadequate and equivocal cytologic diagnoses is appealing, final assessment of the overall cost-effectiveness of HPV testing in combination with cytology for cervical carcinoma screening awaits the results of larger studies that are now underway.

REFERENCES

1. Broder S. The revised Bethesda System for reporting cervical/vaginal diagnoses: report of the 1991 Bethesda Workshop. *JAMA* 1994;267:1992.
2. Kurman RJ, Henson DE, Herbst AL, Noller KL, Schiffman MH. Interim guidelines for management of abnormal cervical cytology. *JAMA* 1994;271:1866–9.
3. Davey DD, Naryshkin S, Nielsen ML, Kline TS. Atypical squamous cells of undetermined significance: interlaboratory comparison and quality assurance monitors. *Diagn Cytopathol* 1994;4:390–6.
4. Wied GL, Bartels PH, Bibbo M, Gupta PK, Gurley AM, Hilgarth M, et al. Computer-assisted quality assurance. *Acta Cytol* 1996;40:1–3.
5. Tawa K, Forsythe A, Cove JK, Saltz A, Peters HW, Watring WG. A comparison of the Papanicolaou smear and the Cervigram: sensitivity, specificity, and cost analysis. *Obstet Gynecol* 1988;71:229–35.
6. Schiffman MH, Sherman ME. HPV testing to improve cervical cancer screening. In: Srivastava S et al., editors. Early detection of cancer: molecular markers. Armonk, NY: Futura Publishing Co., 1994:265–77.
7. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. *J Natl Cancer Inst* 1995;87:796–802.
8. Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB, et al. Epidemiologic evidence that human papillomavirus causes most cervical intraepithelial neoplasia. *J Natl Cancer Inst* 1993;85:958–64.
9. IARC monograph on the evaluation of carcinogenic risk to humans: human papillomaviruses. Volume 64. Lyon, France: IARC, 1995.
10. Sherman ME, Schiffman MH, Lorincz AT, Manos MM, Scott DR, Kurman RJ, et al. Toward objective quality assurance in cervical cytopathology: correlation of cytopathologic diagnoses with detection of high-risk human papillomavirus types. *Am J Clin Pathol* 1994;101:182–7.
11. Cox JT, Lorincz AT, Schiffman MH, Sherman ME, Kurman RJ, Hussain M, et al. Human papillomavirus testing by Hybrid Capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol* 1995;172:946–54.

12. Farthing A, Masterson P, Mason WP, Vousden KH. Human papillomavirus detection by Hybrid Capture and its possible clinical use. *J Clin Pathol* 1994;47:649–52.
13. Hatch KD, Schneider A, Abdel-Nour MW. An evaluation of human papillomavirus testing for intermediate- and high risk HPV types as triage before colposcopy. *Am J Obstet Gynecol* 1995;172:1150–7.
14. Wright TC, Sun XW, Koulos J. Comparison of management algorithms for the evaluation of women with low-grade cytologic abnormalities. *Obstet Gynecol* 1995;85:202–10.
15. Richart R. A modified terminology for cervical intraepithelial neoplasia. *Obstet Gynecol* 1990;75:131–2.
16. Aponte-Cipriani SL, Teplitz C, Rorat E, Savino A, Jacobs AJ. Cervical smears prepared by an automated device versus the conventional method: a comparative analysis. *Acta Cytol* 1995;39:623–30.
17. Bur M, Knowles K, Pekow P, Corral O, Donovan J. Comparison of ThinPrep preparations with conventional cervicovaginal smears: practical considerations. *Acta Cytol* 1995; 39:631–42.
18. Hutchinson MH, Agarwal P, Denault T, Berger B, Cibas ES. A new look at cervical cytology: ThinPrep multicenter trial results. *Acta Cytol* 1992;36:499–504.
19. Wilbur DC, Cibas ES, Merritt S, James LP, Berger BM, Bonfiglio TA. ThinPrep processor: clinical trials demonstrate an increased detection rate of abnormal cervical cytologic specimens. *Am J Clin Pathol* 1994;101:209–14.
20. Herrero R, Schiffman MH, Bratti C, Hildesheim A, Balmaceda I, Sherman ME, et al. Design and methods of a population-based natural history study of cervical neoplasia in a rural province of Costa Rica: the Guanacaste Project. *PAHO J Public Health.* In press.
21. Hussain OAN, Butler EB, Nayagam M, Mango L, Alonzo A. An analysis of the variation of human interpretation: PAPNET a mini-challenge. *Anal Cell Pathol* 1994;6:157–63.
22. Koss LG, Lin E, Schreiber K, Elgert P, Mango L. Evaluation of the PAPNET cytologic screening system for quality control of cervical smears. *Am J Clin Pathol* 1994;101:220–9.
23. Sherman ME, Mango LJ, Kelly D, Paull G, Ludin V, Copeland C, et al. PAPNET analysis of reportedly negative smears preceding the diagnosis of a high-grade squamous intraepithelial lesion or carcinoma. *Mod Pathol* 1994;7:578–81.
24. Schiffman MH, Kiviat NB, Burk RD, Shah KV, Daniel RW, Lewis R, et al. Accuracy and reliability of human papillomavirus DNA testing by Hybrid Capture. *J Clin Microbiol* 1995;33:545–50.
25. Bauer H, Greer C, Manos M. Detection of genital human papillomavirus infection using PCR. In: Herrington CS, McGee JO, editors. Diagnostic molecular pathology: a practical approach. Oxford University Press, 1992:131–52.
26. Manos MM, Ting Y, Wright DK, et al. The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. In: Furth M, Greaves MF, editors. Molecular diagnostics of human cancer. Cold Spring Harbor, NY: Cold Spring Harbor Press, 1989:209–14.
27. Hildesheim A, Schiffman MH, Gravitt P, Glass AG, Greer C, Zhang T, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women in Portland, Oregon. *J Infect Dis* 1994;169:235–40.
28. Morell ND, Taylor JR, Snyder RN, Ziel HK, Saltz A, Willie S. False-negative cytology rates in patients in whom invasive cervical cancer subsequently developed. *Obstet Gynecol* 1982;60:41–5.
29. Gay JD, Donaldson LD, Goellner JR. False-negative results in cervical cytologic studies. *Acta Cytol* 1985;29:1043–6.
30. Kristensen GB, Skyggebjerg K-D, Holund B, Holm K, Hansen MK. Analysis of cervical smears obtained within three years of the diagnosis of cervical cancer. *Acta Cytol* 1990;35:47–50.
31. Hall S, Wu TC, Fields A, Sherman ME, Erozan Y. Suitability of ThinPrep specimens for immunocytochemistry and gene amplification by polymerase chain reaction [abstract]. *Acta Cytol* 1992;35:585.